How to choose the right confocal microscope?

By Alex & Gaëtan

GIGA — GENERAL TIPS & TRICKS

The platform offers a wide range of confocal microscopes, which allow you to perform experiment from basic confocal microscopy to high-end techniques.
These microscopes are accessible after a training dispensed by Alexandre (Alexandre.Hego@uliege.be) or Gaëtan (Gaetan.Lefevre@uliege.be).
Prior to the acquisition, our team can assist you with :

- ☑ Selecting the best microscopy technique
- ☑ Choosing the appropriate microscope
- ☑ Giving advice on sample preparation & labeling strategies
- ☑ Helping you to create settings for your acquisition

Best Practices in Light Microscopy

Optimize sample preparation techniques to minimize autofluorescence, especialy when working with paraformaldehyde (PFA). Autofluorescence can be reduced using methods such as $CuSO_4$, Glycine, TrueBlack, Sudan BlackB, H_2O_2 ...

For immunofluorescent staining, it is always important to use photostable fluorophores, and include negative, positive and isotype controls for each experimental condition.

Any type of support can be used on a confocal microscope. But, when working with magnifications higher than 20x, it is essential to use thin glass supports. A recommended thickness is N°1.5, approximately 0.17mm, which can be achieved with options such as slides with coverslips (1.5), Ibidi slides, 35mm dishes with glass bottoms, or multiwell plates with glass bottoms.

Clean the objective before and after each use of the microscope, even if you use dry objective. A dirty microscope can result in images with lower pixel intensities.

Keep the same parameters across all your images to ensure comparability. This includes using the same laser power, PMT voltage, filter set, objectives, number of pixels, step in Z, pinhole size, etc…

ALWAYS KEEP IN MIND :

You are the only one responsible for your data

Never use the platform's computers as a back-up for your data !

SP5, FV1000 & A1R

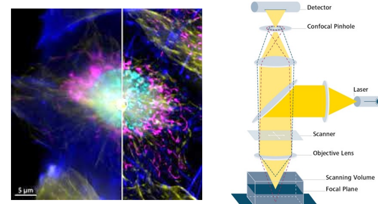
Standard confocal microscopes with 240nm resolution in XY.

Applications :
- 2D, Z-stack (3D), Timelapse, Tilescan
- Colocalisation
- Spheroïds (if thickness thinner than 150 µm)
- Cell biology (morphology, dynamics, structure)

Leica SP5
- Lasers (405, 458, 476, 488, 494, 561, 633nm)
- AOBS (select of specific emmision wavelenght)

Olympus FV1000
- Lasers (405, 458, 488, 515, 561, 633nm)
- FRAP

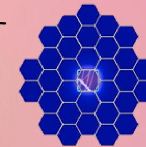
Nikon A1R
- Lasers (401, 488, 561, 639nm)
- Long working distance objectives
- Temperature & CO_2
- Spectral unmixing (separation of overlapping fluorescence signals)



LSM 880 & 980

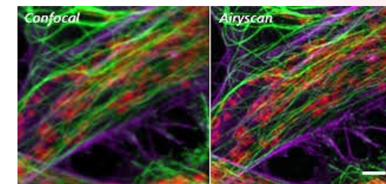
Airyscan technology uses a concentric zone detector, capturing super-resolution information at around 120nm. Its advantage lies in collecting more light across the entire detector zone, enhancing the signal-to-noise ratio and improving image quality compared to standard confocal systems.

Applications :
- As previous
- RNAscope, FRAP, FRET
- Subcellular imaging
- Live cell imaging
- Bacteria



Zeiss LSM 880
- Airyscan 1
- Lasers (355, 405, 458, 488, 514, 561, 633nm)
- Temperature & CO_2

Zeiss LSM 980
- Airyscan 2
- Lasers (405, 488, 561, 639nm)
- Temperature & CO_2
- Spectral unmixing (separation of overlapping flurescence signals) can split up to 6 fluorophores.

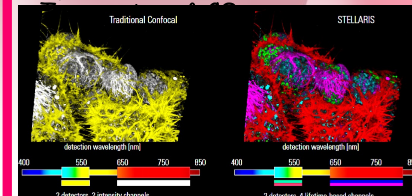


Stellaris 8

The Stellaris 8 has several advantages over a standard confocal. The white light laser (WLL) has 8 simultaneous laser lines, ranging from 440nm to 790nm, for versatile excitation. TauSense is a set of imaging tools that give users instant access to fluorescence lifetime information. This allows you to split spectrally overlapping fluorophores, remove auto-fluorescence etc… Hyd S & X are more sensitive detectors which, in combination with WLL, enable FLIM.

Applications :
- As with the SP5
- FRET, FLIM
- Study microenvironment
- Spectral unmixing (separation of overlapping flurescence signals)
- Multiplexing with up to 10 fluorophores.

Stellaris 8
- Lasers (405nm, WLL)
- AOBS
- Hyd S & X



GIGA Cell Imaging & Flow Cytometry Platform, Uliège, Belgium

https://www.gigaflowcytometry.uliege.be https://www.gigacellimaging.uliege.be